AWARD NUMBER: W81XWH-15-1-0291

TITLE: Localizing and Assessing Amputee Pain with Intense Focused Ultrasound

PRINCIPAL INVESTIGATOR: Pierre D. Mourad

RECIPIENT: University of Washington
Seattle, WA 98195

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
xx-xx-xxxx October 2016	Annual Report	xx-xx-xxxx 15 Sep 2015 - 14 Sep 2016

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Localizing and Assessing Amputee Pain with Intense Focused Ultrasound	13282005I
	5b. GRANT NUMBER W81XWH-15-1-0291
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
Pierre D. Mourad, PhD Email: doumitt@uw.edu	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
Department of Neurological Surgery, University of Washington, Box 356470 Seattle WA 98195-6470	

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Here we propose *exploratory* studies to stimulate subcutaneous pain generators in patients within residual limbs using our existing research image-guided intense focused ultrasound (ig-iFU) system. Our targets for iFU stimulation include neuromas, intact nerves, TMR (targeted muscle reinnervation) sites, and patient-identified 'sensitive areas'. Successful application of iFU to deep, potentially painful tissue should allow us to locate that tissue and compare their relative sensitivity to external stimulation. This would be consistent with our preliminary human data. Also, our rat studies suggest it plausible that painful tissue in humans would require less ultrasound to generate a sensation than surrounding tissue. We specifically anticipate that we will show that TMR sites are less sensitive than neuromas. This and our questionnaire studies will support the view that TMR surgery may reduce amputee pain relative to standard amputation surgery.

15. SUBJECT TERMS
Image-guided intense focused ultrasound (ig-iFU), Intense focused ultrasound (iFU), Targeted muscle Reinnervation surgery (TMR), Limb amputation, Ultrasound

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USARMC
					19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. INTRODUCTION:

Chronic pain is problematic for many amputees. That pain can have many causes, such as buildup of scar tissue at the end of cut nerves that then applies pressure to the nerve ending. In addition, traditional amputation surgery almost always produces a neuroma at the end of the nerve, itself a source of tenderness and pain. A new surgery, called "Targeted Muscle Reinnervation" or TMR surgery cuts the nerve and then implants it into nearby muscle. TMR surgery has anecdotal evidence of reduced pain for amputees relative to standard amputee patients, an important though unstudied finding. Here we seek to address this question directly, through two means. One is use of questionnaires to assess patient's pain, which gives a general sense of the patient's experience of their pain; the other uses image-guided focused ultrasound device (ig-iFU) to directly test the sensitivity of cut nerve endings in residual limbs of amputee patients. To support this, we will work with two, 45-participant cohorts of patients: TMR and standard amputation surgeries. We will, in addition, enroll 45 non-amputee participants as a control cohort. Our ig-iFU device uses ultrasound imaging to locate neuromas, nerves, and tissue, and individual, short pulses of high-intensity ultrasound to stimulate the nerve endings in the residual limbs. In this way we will directly determine which are more sensitive: those of standard amputee patients or of TMR patients. An important outcome of this study will be determination of the relative merits of each surgical procedure as far as their impact on patient pain.

2. KEYWORDS:

- Image-guided intense focused ultrasound (ig-iFU)
- Intense focused ultrasound (iFU)
- Targeted muscle Reinnervation surgery (TMR surgery)
- Limb amputation
- Ultrasound

3. OVERALL PROJECT SUMMARY:

Bulleted summary of tasks completed according to the *Summary of Work*

Research Objective #1: Determine the iFU threshold value required for reliable sensation induction and characterize those sensations, for control tissues within healthy test subjects as well as within individuals with unilateral amputations, with or without TMR.

Task 1. Amend existing human subjects protocol at HMC to include the more extensive studies described here (Q1/12).

- Complete: IRB and military HRPO approval have been obtained. We are currently in the process of obtaining additional IRB and HRPO approval to pre-screen and recruit TMR patients, as well as send a cover letter to all amputee patients in our database.

Task 2. Identify and consent test subjects with unilateral amputations or intact volunteers. (Q2-12/12).

- In progress: Task 3 was completed first, as we were waiting on having a device before consenting volunteers. We now have a database of TMR and regular amputee patients from Dr. Friedly, and will begin posting flyers at two locations. We will begin identifying and consenting healthy volunteers starting in the end of October and early November.

Task 3. Amend our existing ig-iFU device as necessary (Q2-12/12).

- Complete: Figures 1 and 2 show components of our completed ig-iFU device.

Task 4. Image and thereby locate with our ig-iFU device a major peripheral nerve in the appropriate contralateral limb of patients or of controls. (Q2-12/12).

- In progress: Our ultrasonographer has become skilled at using the ig-iFU device to locate major peripheral nerves. This task will continue into year two as we image nerves in our patients and controls.

Task 5. Determine the iFU threshold value for an intact peripheral nerve and record the type and duration of the associated sensations. (Q2-12/12).

- Not yet started

Research Objective #2: Determine the iFU threshold value of target tissue in amputee patients.

Task 6. Identify and consent test subjects with unilateral amputations. (Q2-12/12).
As above in Task 2.

- Not yet started: See task 2. We have a database of TMR and regular amputees. We will initiate the identification and consent process with these cohorts after completion of the research in healthy volunteers.

Task 7. Image, hence locate the neuroma, TMR site, and patient-identified sensitive areas as appropriate, in the patient's residual limb with our ig-iFU device. (Q2-12/12).

- Not yet started

Task 8. Stimulate the neuroma or TMR site, as appropriate, in the patient's residual limb with our ig-iFU device. (Q2-12/12).

- Not yet started

Task 9. Apply questionnaires to patients to assay their pain. (Q2-12/12).

- Not yet started

Task 10: Write up all results for publication and presentation. (Q5-12/12).

- Not yet started

Task 11. Visit Northwestern (Q5-10/12).

- Not yet started

Research objective #3: Develop specifications of a clinical device that embodies TAP.

Task 12. Identify first-order ultrasound protocols and associated devices necessary to TAP (Q9-12/12).

- Not yet started

Summary of Current Objectives

During year one, our goal was to complete milestone one, and begin milestones two and three: obtaining UW and military IRB approval, successfully testing the ig-iFU device on the first 45 test subjects (15 each of standard amputee, TMR, and intact volunteers), and expanding the ig-iFU scope to the entire 135 subjects. These milestones fall under research objectives one and two. For research objective one, the aim is to determine the threshold value of iFU that is required for reliable sensation within control tissue of patients in the three cohorts. This will then be qualitatively and quantitatively recorded. Research objective two builds off of the findings of research objective one to determine the iFU threshold for reliable sensation induction in target tissue within the three cohorts. The results of research objective two will also be qualitatively and quantitatively recorded.

Summary of Progress, Results, and Accomplishments Under Objectives One and Two

We have completed task one by obtaining UW IRB, and military HRPO approval. We have also completed task three, and have developed an ig-iFU machine (Figures 1 and 2).

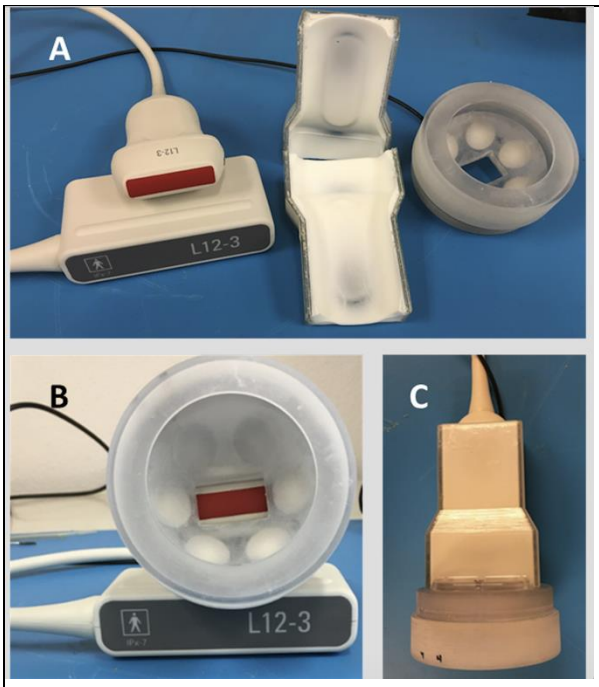

Figure 1
A: Phillips EPIQ L12-3 transducer, next to the housing and iFU transducer
B: Assembled transducer in an axial view
C: Side view of the assembled ig-iFU transducer

Figure 2
The working console of the Philips Epiq with the assembled ig-iFU transducer and gel standoff as it would appear when ready to use.

Task 3, developing and amending our ig-iFU device was completed ahead of task two, in which we identify and consent test subjects with unilateral amputations or intact volunteers, because we felt that it was important to be ready to schedule a research session in a timely fashion after obtaining consent. This would not have been a possibility had we not finished a working model of the ig-iFU device. We are therefore in the initial stages of task two: we have a database of TMR patients that we obtained from our collaborating physician, Dr. Friedly. The research coordinator is preparing to mail IRB-approved flyers to these patients, so that interested patients can be consented. We are also applying with the UW IRB to modify our protocol to allow us to screen and mail flyers to regular, non-TMR patients, as we anticipate the need to reach out to a

broader patient base. This change will likely lengthen the duration of task two, necessitating that it be continued through the beginning of year two.

Task four under research objective one states that we will image and thereby locate with our ig-iFU device a major peripheral nerve in control tissue for either amputees in either group, or control subjects. Task four is scheduled to last until quarter twelve. Although we are still working on task two, in which we recruit and consent test subjects, we have taken steps to train our ultrasonographer to re-producibly image peripheral nerves in healthy tissue. Here, our ultrasonographer used the assembled ig-iFU transducer's imaging capability alone, not its iFU modality, to image our PI's right medial nerve (figure 3).

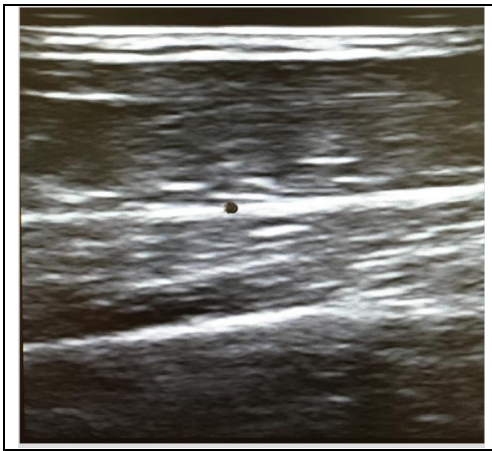

Figure 3:
Image captured by the EPIQ, when coupled to the ig-iFU device. The black dot on the screen represents the calibrated focus for the iFU, though here, iFU was not being used. The thick white line in the center of the screen that is bisected by the dot is the right medial nerve of the PI's arm.

Given that our device has the capability to image at six separate depths, we took focus images with each of the three gels and both of the ig-iFU assemblies. This was accomplished by using a needle hydrophone to locate the center of the ultrasound field (found by correlating the spatial position to the maximum ultrasound intensity), then using the EPIQ to image the location of the tip of the needle hydrophone at this distance. These images will allow us to know the focal depth of the ig-iFU stimulation when we are ready to determine iFU threshold in study participants. One such calibration image is seen in figure 4, where the white grain of rice-sized streak represents the tip of the hydrophone, and the longer line above represents the surface of the gel. In this process, we also made spatial maps of ultrasound intensity.

Figure 4:
Sample calibration image for the ig-iFU device. The circled white line is the tip of the needle hydrophone. This represents the focus of the ig-iFU, and can be superimposed when the device is used to image and stimulate a nerve for precision.

At this point, we have not initiated task five, the last task under research objective one, or tasks 6-9 in research objective two. Note that none of these tasks are scheduled to conclude until quarter twelve. We anticipate these tasks will naturally be completed as soon as we have consented our patients, scheduled their appointments, and completed their 90-minute sessions. This should continue until quarter 12, as expected, therefore we are not behind schedule on their completion.

Actual or anticipated problems or delays and actions or plans to resolve them
Near the end of the fourth quarter of our research effort, our lead engineer accepted a job in the private sector. We have had to transition to a new, but highly capable engineer who completed the final steps of the construction of our ig-iFU device. This delay has slowed down our research efforts. To compensate for this delay, we plan to put in place an accelerated recruitment and study plan over the next year.

Changes in approach and reasons for these changes.
Our initial study design incorporated only one consenter. We anticipate this may lead to delays when this individual is unavailable. In response, we will add Dr. Mourad as a consenter in order to add flexibility and expedite the consent process. We will also add a cover letter from Dr. Friedly to accompany the recruitment flyer in order to provide supplemental information to the candidate test subjects. Both of these changes required minor IRB and HRPO modifications, and approval. We will proceed on these fronts after this has been received, but for the time being, we are able to recruit healthy volunteers (Task 2 in our summary of work). For this reason, we do not anticipate any significant setback.

4. **KEY RESEARCH ACCOMPLISHMENTS:** We have submitted a manuscript to the journal, Pain Management, showing the feasibility of our proposed study. A copy of the manuscript entitled *Intense Focused Ultrasound Preferentially Stimulates Transected Nerves within Residual Limbs*[1] can be found accompanying this report. In addition, the testing, calibration, and finalization of our ig-iFU marks a significant project milestone. Having prepared for in vitro use, we are on the verge of applying it to test subjects.

5. **CONCLUSION:** Year one has laid the groundwork for future research findings. The ig-iFU device that has been developed during this year for use within the project has the potential to streamline the diagnosis of painful tissue deep to the site of limb amputation. It may also elucidate differences in pain sensitivity between traditional limb amputation surgeries, and TMR surgery. The possibility of greater objectivity in pain diagnosis, and in surgery selection indicate that the ig-iFU system may enhance a physician's repertoire, empowering both doctor and patient. These possibilities are especially beneficial to veterans within the amputee community.

   The next tasks in this research to complete form the core of the study: enrolling and consenting 15 amputee patients from each of TMR amputation, traditional amputation, and control patients, finding the iFU threshold stimulation value, and expanding the

study to include the full 45 patients per group. We will begin this process within the next quarter, and expect to finish on schedule by quarter twelve.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Peer-Reviewed Scientific Journals:

a. Manuscript title: *Intense Focused Ultrasound Preferentially Stimulates Transected Nerves within Residual Limbs*[1]
b. Authors: Pierre D. Mourad, Janna L. Friedly, Abbi M. McClintic, Tessa A. Olmstead, John D. Loeser
c. Journal Name: Pain Medicine
d. Editors: TBD
e. Page numbers: TBD
f. Date: TBD

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES: We have developed an ig-iFU machine to simultaneously stimulate specific deep tissue with intense focused ultrasound, while generating a high-resolution ultrasound image. Given favorable study results in localizing and assessing amputee pain, we anticipate that the device has the capability to improve quality of life for amputees. By non-invasively and specifically stimulating and imaging deep residual limb tissue, the device may elucidate generators of pain for amputees. We also hope that it will help to differentiate TMR and traditional amputation surgeries, giving amputees greater latitude.

9. OTHER ACHIEVEMENTS: We have begun to write an NIH R01 motivated by these studies.

10. REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

1. Mourad, Pierre D., Friedly, Janna L., McClintic, Abbi M., Olmstead, Tessa A., Loeser JD. Intense Focused Ultrasound Preferentially Stimulates Transected Nerves within Residual Limbs. *Pain Med*. 2016.

11. APPENDICES: Please see the attached manuscript

Intense focused ultrasound preferentially stimulates transected nerves within residual limbs

Pierre D. Mourad, PhD (1,2), Janna L. Friedly, MD (3), Abbi M. McClintic, BS (1), Tessa A. Olmstead, BS (1), John D. Loeser, MD (1)

(1) Department of Neurological Surgery, University of Washington, Seattle WA

(2) Division of Engineering and Mathematics, University of Washington, Bothell WA

(3) Department of Rehabilitation Medicine, University of Washington, Seattle WA

Pierre D. Mourad

Department of Neurological Surgery

University of Washington

Box 356470

1959 NE Pacific St.

Seattle WA 98195-6470

phone: 206-713-7797; fax: 206-543-8315

email: doumitt@uw.edu

*All of the authors may be reached at this address.

This work received financial support from the Life Sciences Discovery Fund of Washington State, and the Department of Defense

Running title: ultrasound stimulates nerves in residual limbs**.**

Abstract

Objective. Identifying pain generators in tissue deep to the skin can require uncomfortable, complicated, and invasive tests. We describe pilot studies testing the hypothesis that ultrasound image-guided, intense focused ultrasound (ig-iFU) can non-invasively and differentially stimulate the end of transected nerves in the residual limbs of amputee patients.

Design. We applied iFU to the transected nerve ending as individual pulses of length 0.1 seconds using a carrier frequency of 2.0 MHz. After targeting we gradually increased the iFU intensity to reach consistent patient-reported stimulation of the transected nerve ending. We also stimulated the proximal nerve, tissue near the nerve ending, and intact contralateral nerve. We describe the resulting sensations and correlated the results of the test subject's pre-iFU study responses to phantom- and residual-limb pain questionnaires.

Results. iFU intensity values between 16 W/cm^2 and 433 W/cm^2 applied to the transected nerve ending and proximal nerve elicited sensations, including those of the phantom limb, while the same intensity applied to control tissue centimeters away from the nerve ending, or to intact nerve on the contralateral limb, did not. Two out of eleven study participants reported only mild and transient pain created by iFU stimulation. Successful iFU intensity values did not correlate with phantom- and residual-limb pain scores.

Conclusion. Transected nerves had greater sensitivity to iFU stimulation than ipsilateral and contralateral control tissue, including intact nerve. These results support the view

that ig-iFU may one day help physicians identify deep, tender tissue in patients who report experiencing pain.

Keywords: Intense Focused Ultrasound; neuroma; transected nerve; locating deep and tender tissue.

Introduction.

A patient's pain may arise from readily identifiable peripheral sources via nociceptive pathways and/or amplify or primarily occur due to central sensitization. These factors can make pain diagnosis and treatment difficult. As part of a diagnostic armamentarium for either acute or chronic pain, physicians often perform evocative tests such as palpation in addition to imaging in order to try to locate and identify deep potential pain generators. Manual palpation (or its complement, anesthetizing via injection) of deep, potentially tender tissue also involves the intervening, generally superficial tissue, adding complexity to pain diagnosis. In addition, commonly used imaging studies may identify multiple candidate pain generators or find only normal-appearing tissue at the site of sensitivity, also making diagnosis and treatment of pain more difficult. For example, new and evolving bone metastases can create significant pain that may require ablative techniques that target an individual metastasis when bisphosphonate drugs fail (1). Since metastases are easily seen on imaging, but are often asymptomatic (2) imaging alone may not adequately direct treatment. As another example, 50 to 80% of people with amputations experience pain that arises through a combination of peripheral sources such as neuromas as well as peripheral and central sensitization that significantly impacts function and quality of life (3–8) with generally ineffective diagnosis and treatments (9,10). Neuromas are ubiquitous in people with

amputations and appear readily on ultrasound and MR imaging. Determining whether a neuroma actually contributes to a patient's pain, however, requires stimulating the neuroma to see if that reproduces the experienced pain, problematic for deep neuromas and currently impossible without stimulating intervening tissue that may also contribute to the patient's pain.

Therefore, a non-invasive means of stimulating small, deep and potentially tender tissue could significantly improve the ability of a clinician to locate painful tissue, a step towards identifying or ruling out the existence of pathology at the site of tenderness.

Another challenge in the diagnosis of pain is that patients' self-report of pain, while critical to diagnosis and treatment, is subjective and difficult to interpret. Even when physicians can plausibly palpate or inject candidate tender tissue, they cannot easily do so in a manner blinded to the patient. Reliance on patients' self-report of pain remains true during treatment for their pain. This reliance adds to the complexity of pain diagnosis and treatment. For example, in the case of chronic low back pain, pain is strongly associated with psychosocial factors that include depression, fear avoidance, catastrophizing, insurance status, among other factors (11), the patient's perceived attitude of the medical provider and their preferred treatments (12) and the early use of MRI imaging (13). Therefore, there exists a need to assess the tenderness of deep tissue associated with a patient's pain upon presentation as well as during pain treatment, especially a non-invasive method applicable to the patient in a double-blinded fashion.

Use of intense focused ultrasound (iFU) may represent a viable method for identifying deep painful tissue. Our previous work shows that iFU can elicit differential responses to

stimulation in diffusely inflamed and neuropathic rat paws (14–17). In addition, several researchers have shown that sufficiently intense iFU can generate sensations in healthy test subjects when applied to superficial tissue (18–21). Moreover, we have applied it successfully to identify focal and subcutaneous sources of shoulder pain in humans (22). Finally, in anticipation of the present study, we have applied iFU stimulation to a rat model of a transected nerve that formed a palpable and subcutaneous neuroma (16). We showed that iFU could stimulate the neuroma while the rats were lightly anesthetized, eliciting a motor response to their stimulation, while the same intensity of ultrasound applied to control tissue failed to induce a motor response.

Together, these results motivate the present study, which seeks to test the hypothesis that image-guided iFU can non-invasively stimulate transected nerve endings in the residual limbs of amputee patients who had undergone either standard amputation surgery or targeted nerve implantation, such that the stimulation differentiates the transected nerve ending from control tissue.

2. Materials and Methods

Patient population.

Our human subject study was approved by the Institutional Review Board of the University of Washington. We recruited study participants in our study from two groups of patients: those who had undergone lower limb amputation surgery using either standard techniques, where the transected nerve ending lies proximal to the end of the residual limb in soft tissue, or a targeted nerve implantation (TNI) technique (23). Briefly, TNI consists of implanting the transected nerve ending into a secondary motor nerve point in a surgically denervated muscle at the time of amputation or during revision surgery.

Participants were recruited via flyers at the Harborview Medical Center Amputation Clinic. Inclusion criteria were: > 6 months since lower limb amputation (transtibial, knee disarticulation or transfemoral), and 18-75 years of age. Exclusion criteria were: current pressure ulcers, rashes, or open skin over residual limb, history of skin grafting or burns on residual limb, history of diabetes mellitus, cognitive or communication impairments that would impede participation in the testing procedures, history of muscle or nerve disease, including peripheral vascular disease, and evidence of alcohol or illicit drug use. We conducted studies on four TNI patients and seven standard amputation patients.

Ultrasound device.

Our iFU system consisted of a portable diagnostic ultrasound imaging machine connected to an intense focused ultrasound stimulation transducer, hence called an image-guided iFU (ig-iFU) system. Figure 1 shows a schematic of the device and of its application. We described it in detail in Gellhorn et al. (2015) (22), so we include here only a summary description here.

Ultrasound device – iFU transducers. We created five 2.0 MHz iFU transducers, each with foci at different depths relative to the skin surface (0.4 cm, 1.3 cm, 2.45 cm, 2.75 cm, and 3.0 cm). For a given patient, we used one transducer to deliver individual single bursts of ultrasound lasting 0.1 seconds, driven by a power amplifier controlled by two function generators. Input voltage, translated into spatial and temporal average intensity, was calibrated in advance of experiments using a hydrophone in a water tank, and was monitored during the experiment using an oscilloscope. Full details regarding calibration can be found in Gellhorn et al. (2015) (22).

Ultrasound device – imaging system. Ultrasound image guidance was provided by a portable Sonosite M-Turbo ultrasound machine with a 13-6 MHz linear transducer whose imaging plane contained the iFU focus as verified by an ultrasound needle hydrophone. Depths of the iFU

transducers were marked on the screen of the Sonosite, such that we could see on the active ultrasound images the target of iFU stimulation.

Ultrasound device – integrated ig-iFU system. The imaging transducer was mounted within a custom housing that screwed onto the iFU transducers. This allowed us to image through a hole in the center of the iFU transducers such that the imaging plane aligned directly with the iFU focus.

Study procedures.

Pain questionnaires. After successful consent, all study participants completed three pain questionnaires. The first questionnaire (24) – the Leeds assessment of neuropathic symptoms and signs (LANSS) – yielded a composite pain score on a scale of 0 to 24 such that scores above 12 pointed to the likelihood of neuropathic pain. Each participant also reported the intensity of their phantom limb pain and of their pain associated with the residual limb itself as experienced over the last 24 hours (numeric pain rating scale from 0='no pain' to 10='pain as bad as you can imagine').

Initial ultrasound imaging and manual palpation. After a given study participant completed their questionnaires, the physician palpated areas in the distal portion of their residual limb to identify a region that contained tender tissue. Next, the sonographer imaged the participant's residual limb using only the ultrasound imaging transducer, in order to identify anatomical structures of interest within the tender region, always the transected nerve ending, with or without an observable neuroma (Figure 2A). The location and depth of the tender sites were noted to facilitate subsequent iFU stimulation with the appropriate iFU transducer under ultrasound image guidance. With regard to the contralateral limb, the sonographer imaged the major nerve that corresponded to the target nerve in the ipsilateral limb, recording the location

and depth as above. The physician did not, however, palpate the nerve in the contralateral limb.

iFU stimulation of targets in the residual limb. After the initial exploratory imaging described above, we selected in a serial fashion the target tissue (the transected nerve ending with or without an identifiable neuroma), then assembled the ig-iFU system using the iFU transducer with the appropriate depth of stimulation. Using the ig-iFU system we then re-located the target tissue site via ultrasound imaging and aligned the focus of the iFU transducer with the target (Figure 2B). We then applied or sham applied iFU in individual 0.1 s bursts to the target tissue in a manner blinded to the study participant. We started with a low iFU intensity value (16 W/cm^2). After each actual or sham application, we asked the participants if they felt any sensations associated with the application. If they did not feel any sensation with the iFU application, we increased the intensity and tried again. If they did indicate that they felt the stimulation, we repeated the iFU stimulation procedure using the same iFU intensity to verify the sensation. If they did not feel a sensation this second time we increased the intensity of iFU stimulation and tried again. Once a participant reported two consecutive sensations (our definition of a 'reliable' sensation) at the same intensity, we did not increase the intensity of iFU any further for that target. We define this intensity as the iFU threshold intensity value. For sites where we were unable to elicit a sensation as we raised the iFU intensity, we stopped our studies when we reached 1032 W/cm^2, the maximum output of the device. We then moved the focus of the ig-iFU device to control tissue ~1cm superficial to the transected nerve ending, applying actual iFU stimulation to it with the same intensity that generated a sensation when applied to the transected nerve ending, or to the maximum intensity of the device, as appropriate. We then applied that same intensity of iFU to the proximal portion of nerve anatomically associated with the transected nerve ending, again asking about any sensations experienced by the patient due to iFU stimulation.

Finally, when not constrained by patient fatigue, we repeated the entire iFU threshold determination process for the corresponding nerve in the participant's intact, contralateral limb.

Results

Here we report our results as individual case studies for each study participant; see Table 1 for a summary of these results and Figure 3, which gives the correlation of the observed iFU intensity threshold value with each of the phantom limb pain and residual limb pain scores.

TNI patients

In all four cases we identified a neuroma and associated proximal nerve near the site of manual tenderness with diagnostic ultrasound imaging. In two of the four cases iFU stimulation of an intact nerve contralateral to the transected nerve ending did not elicit any sensations. We did not test the contralateral nerve in the other two patients.

P1 (transtibial)

Participant P1 had TNI revision surgery approximately five years before the study in which the superficial and deep peroneal neuromas were excised and the nerve endings implanted into a motor point of the anterior tibialis muscle. One TNI site (with neuroma) was visible via ultrasound imaging and was selected as the target for iFU application. The iFU threshold intensity value for the neuroma was 187 W/cm^2 as it was for the proximal nerve. The participant reported sensations of a “light feather feeling on the surface, a slight electrical shock, and light local and transient pain.” We did not test the contralateral nerve.

P2 (transtibial)

Participant P2 had TNI revision surgery five years before the ig-iFU study, in which the sciatic neuroma was excised and the remaining nerve implanted in a motor point in the hamstring muscle. The participant reported spontaneous phantom sensations as well as intermittent pain and muscle cramps in the residual limb before the study. We found two neuromas, each with an associated proximal nerve as shown by ultrasound imaging.

We applied iFU to each neuroma and to the associated proximal nerve in increasing amounts until we reached the upper limit of the device (1032 W/cm^2), without, however, eliciting any sensations. Also, iFU stimulation up to the upper limit of the device did not generate a sensation in the intact nerve in the contralateral limb.

P3 (transtibial)

Participant P3 underwent TNI revision surgery one year prior to the iFU study, in which the peroneal and tibial neuromas were excised and the nerve endings were each transferred to separate motor points in the hamstring. The participant reported background phantom limb sensations upon manual stimulation of the hamstring. We identified two TNI sites, where each upon ultrasound imaging showed a neuroma and associated proximal nerve. One TNI site and associated nerve were sensitive to iFU applied at the same threshold intensity value of 16 W/cm^2. The other TNI site and associated nerve were sensitive to iFU applied at a threshold intensity value of 66 W/cm^2. For both neuromas and associated nerves, the participant reported feeling after iFU stimulation a “heartbeat like” sensation that lasted for approximately one minute as well as a tingly feeling of the toes associated with their missing limb when the neuromas were stimulated. In addition, when iFU (again, at 66 W/cm^2) was applied to the nerve associated with the second TNI site the participant reported a light burning sensation in the missing big toe, tingling associated with all missing toes as well as the bottom of the foot, and felt sharp but transient pain. iFU stimulation up to the limit of the device did not generation a sensation when applied to the intact nerve in the contralateral limb.

P4 (transtibial)

Participant P4 underwent TNI revision surgery approximately two years prior to the iFU study, in which the tibial and peroneal nerves were transferred to separate motor points in the hamstring. Using ultrasound imaging we identified two neuromas, one at each TNI site, and the associated nerves. The participant reported pain to the touch of the area above one neuroma so we elected to test only the other site. At this site, the participant had the same iFU threshold stimulation intensity values at the neuroma and nerve (16 W/cm^2) and reported sensations that were short, transient 'shock-like' feelings without, however, experiencing actual pain. We did not test the contralateral, intact nerve.

Standard amputation patients

In three out of seven cases we identified neuromas via ultrasound imaging at the site of palpable tenderness at the transected nerve ending. For these cases we determined the iFU threshold intensity value for the neuroma followed by the proximal nerve. In the other four cases we found a transected nerve ending only; here we determined the iFU threshold intensity value for the nerve ending itself, specifically at a point that offered an unambiguous imaging target. We sought to identify the iFU threshold intensity value for the contralateral nerve in five of these patients. For four out of five experiments, iFU stimulation of intact nerve contralateral to the transected nerve ending did not elicit any sensations.

P5 (transtibial)

Participant P5 had no subsequent revision surgeries following amputation. We identified two neuromas and each of their proximal nerves via ultrasound imaging. We observed an iFU threshold stimulation intensity value at 433 W/cm^2 for one of the neuromas and

associated nerve**.** We did not test the other neuroma. We also applied iFU to a nerve on the contralateral, intact leg. The participant was not sensitive to stimulation of this nerve up to the maximum intensity we could apply.

P6 (transtibial)

Participant P6 underwent a vein graft surgery six years before the study and had significant scar tissue around the surgery site. Within the residual limb, we found the transected nerve ending without identifying a neuroma via ultrasound imaging. The iFU threshold stimulation intensity value for this nerve ending was also 433 W/cm^2. The participant described the sensations as a ‘pinprick’ and a ‘muscle twitch’ together on the side of the residual limb. We did not test the sensitivity of the contralateral nerve to iFU stimulation.

P7 (transtibial)

Participant P7 had no revision surgeries after amputation. The participant had a history of pain and may have taken pain medications at the time of the study. We identified one neuroma and the associated proximal nerve via ultrasound imaging. The iFU threshold stimulation intensity for the neuroma was 16 W/cm^2 and the participant reported feeling a missing toe; the proximal nerve was sensitive as well, at the same intensity value as for the neuroma. We did not test the sensitivity of the contralateral nerve.

P8 (transtibial)

Participant P8 had no revision surgeries following amputation. We identified the sciatic nerve ending via ultrasound imaging; we did not find a neuroma. The iFU threshold intensity value for the transected nerve was 64 W/cm^2. The participant reported a short sensation on the skin surface. The participant also reported a warming and tingling

feeling and stripes of “energy” running parallel on the residual limb. We also applied iFU to a nerve on the contralateral, intact leg. The participant was not sensitive to stimulation of this nerve up to the maximum intensity we could apply.

P9 (knee disarticulation)

Participant P9 had no revision surgeries following surgery. We identified the transected sciatic nerve ending via ultrasound imaging; we did not find a neuroma. The iFU threshold stimulation intensity value for the nerve ending was 258 W/cm^2. The participant reported a tingle associated with the missing ankle, which worked its way up to the missing calf, lasting approximately one minute. We also applied iFU to a nerve on the contralateral, intact leg. The participant was not sensitive to stimulation of this nerve up to the maximum intensity we could apply.

P10 (transtibial)

Participant P10 had no revision surgeries following amputation. We identified a neuroma and the associated proximal nerve via ultrasound imaging. The iFU threshold stimulation intensity value at the neuroma was 65 W/cm^2. The participant reported a warm feeling and a twitch in the phantom foot and toes. We were not able to elicit sensations by stimulating the proximal nerve itself, however. We also applied iFU to a nerve on the contralateral, intact leg. The participant was not sensitive to stimulation of this nerve up to the maximum intensity we could apply.

P11 (transfemoral)

Participant P11 had no revision surgeries following amputation. We identified a transected nerve ending in the residual limb with ultrasound imaging but could not find a neuroma. The iFU threshold stimulation intensity value at the nerve was 16 W/cm^2. The

participant described the sensation as “zingers” in the phantom heel. We applied iFU to a major nerve in the contralateral, intact leg. In contrast to all other study participants, this participant reported a sensation in this nerve due to iFU stimulation, specifically a single sensation of a slight tingle down the knee at an iFU threshold stimulation intensity value of 240 W/cm^2, which we could not reproduce.

Pain scores and their relation to iFU stimulation intensity value

The LANSS composite pain score measures the overall neuropathic pain level experienced by each study participant. All except two participants had LANSS scores above 12, indicating the likely presence of neuropathic pain in the majority of our participants. iFU threshold stimulation intensity values for all patients trended inversely but without statistical significance with the phantom limb pain experienced by the participants over the last 24 hours (Figure 3A; R^2 = 0.18, p > 0.05). Similarly, iFU threshold stimulation intensity values for all patients trended inversely but without statistical significance with the residual limb pain experienced by the participants over the last 24 hours (Figure 3A; R^2 = 0.14, p > 0.05). iFU threshold stimulation intensity values for TNI study participants did have a statistically significant slope as measured against each of phantom limb and residual limb pain scores (respectively: R^2 = 0.68 and R^2 = 0.55 with p < 0.05; regression lines not shown). In contrast the same analysis applied to standard amputation study participants did not show a meaningful trend (respectively: R^2 = 0.008 and R^2 = 0.003 with p > 0.05; regression lines not shown).

4. Discussion

In this study we sought to determine if intense focused ultrasound (iFU) could stimulate nerve tissue deep to the skin (a transected nerve ending with or without an observable neuroma in a residual limb), and whether or not nerve tissue in residual limbs is more or less sensitive to iFU stimulation than control tissue. Our results demonstrated that sufficient iFU (the 'iFU threshold intensity value'), applied to deep and focal nerve tissue generated discernable sensations, including phantom limb sensations. Moreover, iFU stimulation of control tissue for a given patient (tissue within a centimeter of the neuroma or nerve ending that lay between the iFU source and its target; a major nerve in the contralateral limb) with the same iFU threshold stimulation intensity value for that patient did not induce within them a discernable sensation. Moreover, for contralateral and intact nerves, we could not identify an iFU threshold stimulation intensity value in 6/7 cases (tested up to 1032 W/cm^2).

As a secondary hypothesis, we anticipated that across patients, the iFU threshold intensity values would scale inversely with patient's residual limb and phantom limb pain scores. We observed only a weak and non-statistically significant inverse correlation for our entire cohort of study participants, leaving this hypothesis falsified thus far.

Potential clinical implication of iFU stimulation.

Existing methods for characterizing painful tissue, such as manual palpation, thermodes, lasers, or Peltier devices, stimulate superficial tissue only, or superficial and deep tissue simultaneously. In contrast, iFU can stimulate focal and deep anatomical structures without, moreover, stimulating the intervening tissue, a potentially useful difference in the clinic setting. When coupled with imaging, the clinician could use iFU to more readily identify deep and tender tissue, a first step in the diagnosis and treatment of patient's pain. More refined targeting via iFU

could in turn motivate application of more refined diagnostic and/or imaging techniques in order to identify the presence and type of peripheral pathology at the site of tenderness. Such identification would then allow for more targeted peripheral interventions such as injections or surgery if warranted. In the absence of identified peripheral pathology after this extra diagnostic attention, the physician may more readily move to treatment of potential central contributions to the patient's pain.

Neuroma-related pain and phantom limb pain (PLP) pose particular diagnostic and treatment challenges as it can be difficult clinically to determine if the pain is primarily peripherally-generated, centralized or a combination. iFU is a potential tool to both identify specific peripheral tissues that are pain generators (i.e. neuromas) and to track the efficacy of pain treatments. Pain management is especially problematic for patients with amputation, as neuromas and PLP cause loss of function and reduced quality of life for most adults with amputation. We have shown previously (15) that iFU stimulation threshold values track thermal measures of diurnal variations in inflammatory pain in a rat model, consistent with the idea that through use of ig-iFU a medical provider may have the ability to track changes in a patient's pain during treatment. With this in mind, we hypothesize that an increase in iFU stimulation value over time for a given patient may indicate effective pain management, an especially useful finding since clinicians can apply iFU in a way blinded to the patient (and we assert, blinded to the physician themselves, through design of an appropriate user interface). Interestingly, though not yet the focus of formal study, after TMR (targeted muscle reinnervation surgery, quite similar to the TNI procedure – 25) patients appear to report less pain than standard amputee patients. Our pilot results suggest it possible to use ig-iFU to quantify the sensitivity of transected nerve endings that arise after standard amputation relative to the corresponding nerves at the implantation site of TMR patients, a focus of future study.

It is also possible that the utility of iFU may extend to conditions other than neuromas associated with amputation such as chronic low back pain. It is well known that current imaging techniques identify abnormalities that often do not correlate with back pain (26) and the presence of abnormal findings on imaging often leads to ineffective or even counter-productive treatments such as surgery (27). Use of iFU stimulation could allow clinicians to rule out the presence of specific peripheral pain-generating tissue and may therefore prevent unnecessary surgical interventions. Instead, iFU may help clinicians to more readily attend to central contributors to pain (28,29) rather than peripheral sources of pain: they may therefore more readily prescribe centrally acting medications, meditation, spinal cord stimulation, psychotherapy, continued watchful waiting, among other choices (28).

Future research

Future studies might consider additional study of the mechanisms by which iFU stimulation may generate sensations. The choices we made of pulse duration and transducer frequency used in this study had their motivation in our existing work (17) where we applied a single pulse of iFU of with duration of 100 ms to surgically created neuromas in rat legs. Other studies (18,19,21,30) have also investigated sensation induction by single pulses of iFU with a range of ultrasound frequencies (0.3 – 5.0 MHz) and duration (5 – 100 ms). By using a single short (100 ms) pulse we sought to activate mechanoreceptors, which the literature argues are activated upon ultrasound stimulation (19,30,31). Future studies should consider application of individual and longer pulses, as applied to a neuropathic rat model by Tych et al (2013)(14). In this way one could refine the study of the physical mechanisms by which ultrasound may generate sensations, since a longer pulse may generate heat and activate thermoreceptors at the same time as mechanoreceptors. In addition, future studies might also apply multiple short pulses in rapid succession, to study their potential to induce temporal summation and windup, as suggested by Wright et al (2002)(20) and further explored by McClintic et al (2013a)(16).

Finally, in the present preliminary research, we observed only a weak and statistically insignificant inverse correlation between iFU stimulation intensity value and each of residual limb pain score and phantom limb pain score for our entire cohort. Interestingly, though quite preliminarily given our patient numbers, we observed divergent results between the TNI and standard amputee study participants. We need to perform additional studies with greater participant numbers to determine if our cohort-based results are intrinsic to iFU stimulation or simply a matter of low patient numbers.

Limitations

One initial goal of this study was to explore the idea that targeted nerve implantation surgery could help reduce neuroma formation and therefore help to reduce amputee pain. Unfortunately, in our small cohort, all of the patients who underwent TNI surgery and participated in our study also had an observable neuroma as did 3/7 of the study participants who underwent standard amputation. We were not, therefore, able to make a meaningful comparison between tissue sensitivity associated with these different surgical procedures, a goal for future studies.

We were also not able to test the sensitivity to iFU stimulation of a major nerve in the intact contralateral limbs of all of our study participants due to patient fatigue. Future studies should consider determination of the stimulation threshold of intact nerves in healthy controls versus amputee patients.

In addition, our current studies allowed for blinding of the patient to iFU stimulation, but not the individual delivering the stimulation. We will rectify this in future research through modification of our device to test the usefulness of delivering iFU stimulation in a double-blinded fashion.

5. Conclusion

This study builds on our previous work that studied iFU stimulation of neuromas in a rat model (17). Here, we have conducted a preliminary study of ig-iFU applied to the transected nerve endings of two cohorts of patients with lower extremity amputations – those that have undergone a standard amputation and those that have undergone targeted nerve implantation. We found that the transected nerves in the amputated limbs were more sensitive to iFU stimulation than both local control tissue and the corresponding major nerve in the intact limbs of the same participants. Additionally, we were able to image those targets while performing iFU application, showing successful use of iFU under image guidance.

We have therefore demonstrated the feasibility of non-invasively stimulating transected nerve endings using intense focused ultrasound under image guidance. Future work will explore the potential clinical usefulness of this new means of identifying deep, focal and tender tissue.

ACKNOWLEDGEMENTS

This work received financial support from the Life Sciences Discovery Fund of Washington State. We would like to thank Kate Sweeney for her invaluable help in creating Figure 1. We also thank Doug Smith MD for his surgical acumen as applied to our study participants.

REFERENCES

1. Mercadante S, Fulfaro F. Management of painful bone metastases. Curr Opin Oncol. 2007 Jul;19(4):308–14.
2. Requarth J. Image-Guided Palliative Care Procedures. Surg Clin North Am. 2011;91(2):367–402.
3. Weinstein SM. Phantom limb pain and related disorders. Neurol Clin. 1998;16(4):919–35.
4. Ebrahimzadeh MH, Rajabi MT. Long-term Outcomes of Patients Undergoing War-related Amputations of the Foot and Ankle. J Foot Ankle Surg. 2007;46(6):429–33.
5. Ehde DM, Czerniecki JM, Smith DG, Campbell KM, Edwards WT, Jensen MP, et al. Chronic phantom sensations, phantom pain, residual limb pain, and other regional pain after lower limb amputation. Arch Phys Med Rehabil. 2000;81(8):1039–44.
6. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom Pain, Residual Limb Pain, and Back Pain in Amputees: Results of a National Survey. Arch Phys Med Rehabil. 2005;86(10):1910–9.
7. Ketz AK. The Experience of Phantom Limb Pain in Patients With Combat-Related Traumatic Amputations. Arch Phys Med Rehabil. 2008;89(6):1127–32.
8. Reiber GE, McFarland L V., Hubbard S, Maynard C, Blough DK, Gambel JM, et al. Servicemembers and veterans with major traumatic limb loss from Vietnam war and OIF/OEF conflicts: Survey methods, participants, and summary findings. J Rehabil Res Dev. 2010;47(4):275.

9. Flor H. Phantom-limb pain: characteristics, causes, and treatment. Lancet Neurol. 2002;1(3):182–9.

10. Nikolajsen, L., Jensen TS. Textbook of Pain. McMahon, S.B., Koltzenberg M, editor. Elsevier; 2006.

11. Rodeghero JR, Cook CE, Cleland JA, Mintken PE. Risk stratification of patients with low back pain seen in physical therapy practice. Man Ther. 2015;20(6):855–60.

12. Loeser JD, Cahana A. Pain Medicine Versus Pain Management. Clin J Pain. 2013 Apr;29(4):311–6.

13. Mccullough BJ, Comstock BA, Deyo RA, Kreuter W, Jarvik JG. Major Medical Outcomes With Spinal Augmentation vs Conservative Therapy. JAMA Intern Med.

14. Tych RE, Gofeld M, Jarvik JG, Kliot M, Loeser JD, McClintic AM, et al. Neuropathic Tissue Responds Preferentially to Stimulation by Intense Focused Ultrasound. Ultrasound Med Biol. 2013;39(1):111–6.

15. Garcia JD, Gofeld M, Ray Illian P, Loeser JD, Kliot M, McClintic AM, et al. Intense focused ultrasound as a potential research tool for the quantification of diurnal inflammatory pain. Ultrasonics. 2013;53(1):84–9.

16. McClintic AM, Dickey TC, Gofeld M, Ray Illian P, Kliot M, Kucewicz JC, et al. Rapid ultrasonic stimulation of inflamed tissue with diagnostic intent. J Acoust Soc Am. 2013;134(2):1521.

17. McClintic AM, Garcia JB, Gofeld M, Kliot M, Kucewicz JC, Loeser JD, et al. Intense focused ultrasound stimulation can safely stimulate inflamed subcutaneous tissue and assess allodynia. J Ther Ultrasound. 2014;2(1):8.

18. Dalecki D, Child SZ, Raeman CH, Carstensen EL. Tactile perception of ultrasound. J Acoust Soc Am. 1995 May;97(5 Pt 1):3165–70.
19. Gavrilov LR, Tsirulnikov EM, Davies I a. I. Application of focused ultrasound for the stimulation of neural structures. Ultrasound Med Biol. 1996;22(2):179–92.
20. Wright A, Graven-Nielsen T, Davies II, Arendt-Nielsen L. Temporal summation of pain from skin, muscle and joint following nociceptive ultrasonic stimulation in humans. Exp Brain Res. 2002 Jun 23;144(4):475–82.
21. Dickey TC, Tych R, Kliot M, Loeser JD, Pederson K, Mourad PD. Intense Focused Ultrasound Can Reliably Induce Sensations in Human Test Subjects in a Manner Correlated With the Density of Their Mechanoreceptors. Ultrasound Med Biol. 2012;38(1):85–90.
22. Gellhorn AC, Gillenwater C, Mourad PD. Intense Focused Ultrasound Stimulation of the Rotator Cuff: Evaluation of the Source of Pain in Rotator Cuff Tears and Tendinopathy. Ultrasound Med Biol. 2015;41(9):2412–9.
23. Pet MA, Ko JH, Friedly JL, Mourad PD, Smith DG. Does targeted nerve implantation reduce neuroma pain in amputees? Clin Orthop Relat Res. 2014 Oct;472(10):2991–3001.
24. Bennett M. The LANSS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. Pain. 2001;92(1):147–57.
25. Kuiken TA, Marasco PD, Lock BA, Harden RN, Dewald JPA. Redirection of Cutaneous Sensation from the Hand to the Chest Skin of Human Amputees with Targeted Reinnervation. Proc Natl Acad Sci U S A. 2007;104(50):20061–6.
26. Jarvik, Jeffrey G., Deyo R. Diagnostic evaluation of low back pain with emphasis

on imaging. Ann Intern Med. 2002;(137):586–97.

27. Chou R, Loeser JD, Owens DK, Rosenquist RW, Atlas SJ, Baisden J, et al. Interventional Therapies, Surgery, and Interdisciplinary Rehabilitation for Low Back Pain. Spine (Phila Pa 1976). 2009 May;34(10):1066–77.

28. Dworkin RH, O'Connor AB, Kent J, Mackey SC, Raja SN, Stacey BR, et al. Interventional management of neuropathic pain: NeuPSIG recommendations. PAIN®. 2013;154(11):2249–61.

29. Fishbain DA, Cole B, Lewis JE, Gao J. What Is the Evidence that Neuropathic Pain Is Present in Chronic Low Back Pain and Soft Tissue Syndromes? An Evidence-Based Structured Review. Pain Med. 2014 Jan 1;15(1):4–15.

30. Gavrilov LR, Gersuni G V., Ilyinski OB, Tsirulnikov EM, Shchekanov EE. A study of reception with the use of focused ultrasound. I. Effects on the skin and deep receptor structures in man. Brain Res. 1977;135(2):265–77.

31. Gavrilov LR, Gersuni G V., Ilyinsky OB, Tsirulnikov EM, Shchekanov EE. A study of reception with the use of focused ultrasound. II. Effects on the animal receptor structures. Brain Res. 1977;135(2):279–85.